

Amendments to the Specification:

Please **delete** the following paragraph inserted in the amendment dated December 29, 2003 at page 7, line 18.

--Figure 5 shows an exemplary Pvs28-Pvs25 fusion protein polypeptide of the invention (SEQ ID NO:5). GGGPGGG linker sequence = SEQ ID NO:15.--

Please **replace** the paragraph at **page 7, lines 10-11**, with the following:

--Figure 5 shows an exemplary Pvs28-Pvs25 fusion protein polypeptide of the invention (SEQ ID NO:5). GGGPGGG linker sequence = SEQ ID NO:15.--

Please **delete** the following paragraph inserted in the amendment dated December 29, 2003 at page 7, line 20.

--Figure 6 shows exemplary constructs and recombinant proteins encoded by these constructs (Pvs25 = SEQ ID NO:16; Pvs28 = SEQ ID NO:17; Pvs28Q130 = SEQ ID NO:18; Pvs28NCR = SEQ ID NO:19), including Pfs25 Pvs25, Pvs28, and partially deglycosylated Pvs28 (having a glutamine residue, rather than asparagine, at amino acid residue number 130).--

Please **replace** the paragraph at **page 7, lines 12-14**, with the following:

--Figure 6 shows exemplary constructs and recombinant proteins encoded by these constructs (Pvs25 = SEQ ID NO:16; Pvs28 = SEQ ID NO:17; Pvs28Q130 = SEQ ID NO:18; Pvs28NCR = SEQ ID NO:19), including Pfs25 Pvs25, Pvs28, and partially

deglycosylated Pvs28 (having a glutamine residue, rather than asparagine, at amino acid residue number 130).--

Please **delete** the following paragraph inserted in the amendment dated December 29, 2003 at page 26, line 15.

--The fusion proteins optionally includes additional features such as a flexible linker between Pvs25 and Pvs28 domains. The linkers can facilitate the independent folding of the Pvs25 and Pvs28 proteins. Preferred flexible linkers are amino acid subsequences which are synthesized as part of a recombinant fusion protein. In one embodiment, the flexible linker is an amino acid subsequence comprising a proline such as Gly₃-Pro-Gly₃ (SEQ ID NO:15). In other embodiments, a chemical linker is used to connect synthetically or recombinantly produced Pvs25 and Pvs28 subsequences. Such flexible linkers are known to persons of skill in the art. For example, poly(ethylene glycol) poly(ethylene glycol) linkers are available from Shearwater Polymers, Inc. Huntsville, Alabama. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.--

Please **replace** the paragraph at **page 25, lines 3-12**, with the following:

--The fusion proteins optionally includes additional features such as a flexible linker between Pvs25 and Pvs28 domains. The linkers can facilitate the independent folding of the Pvs25 and Pvs28 proteins. Preferred flexible linkers are amino acid subsequences which are synthesized as part of a recombinant fusion protein. In one embodiment, the flexible linker is an amino acid subsequence comprising a proline such as Gly₃-Pro-Gly₃ (SEQ ID NO:15). In other embodiments, a chemical linker is used to connect synthetically or recombinantly produced Pvs25 and Pvs28 subsequences. Such flexible linkers are known to persons of skill in the art. For example, poly(ethylene glycol) poly(ethylene glycol) linkers are available from Shearwater

Polymers, Inc. Huntsville, Alabama. These linkers optionally have amide linkages, sulphydryl linkages, or heterofunctional linkages.--

Please **delete** the following paragraph inserted in the amendment dated December 29, 2003 at page 27, line 11.

--An exemplary fusion of Pvs25 to Pvs28 by a flexible linker is represented by the polypeptide of Figure 5, SEQ ID NO:5, whose individual domains are:

a Pvs25 sequence (with or without a signal sequence or anchor):

AVTVDTICKNGQLVQMSNHFKCMCNEGLVHLSENTCEEKKETLGKACGEFGQCI
ENPDPAQVNMYKCGCIEGYTLKEDTCVLDVCQYKNCGESGECIVEYLSEIQSAGCSCAI
GKVPNPEDEKKCTKGETACQLKCNTDNEVCKNVEGVYKCQCMEGFTFDKEKNVCLS
(SEQ ID NO:20);

with a flexible linker, *e.g.*: GGGPGGG (SEQ ID NO:15); and

a Pvs28 sequence (with or without signal sequence or anchor):

AKVTAETQCKNGYVVQMSNHFECKCNDGFVMANENTCEEKRDCNPQNVNKNCGY
AVCANTRMNEERALRCGICLGYTVMNEVCTPNKCNGLCGKGKICLDPANVNSTM
SCNIGTTLDESKCGKPGKTECTLKCKANECKETQNYYKCVAKGSGGEGSGGGEGSGG
EGSGGEGSGGEGSGGDTGAAYSLMN **(SEQ ID NO:21)**--

Please **replace** the paragraph at **page 25, line 32 through page 26, line 10**, with the following:

--An exemplary fusion of Pvs25 to Pvs28 by a flexible linker is represented by the polypeptide of Figure 5, SEQ ID NO:5, whose individual domains are:

a Pvs25 sequence (with or without a signal sequence or anchor):

AVTVDTICKNGQLVQMSNHFKCMCNEGLVHLSENTCEEKKETLGKACGEFGQCI
ENPDPAQVNMYKCGCIEGYTLKEDTCVLDVCQYKNCGESGECIVEYLSEIQSAGCSCAI

GKVPNPEDEKKCTKTGETACQLKCNTDNEVCKNVEGVYKCQCMEGFTFDKEKNVCLS
(SEQ ID NO:20);

with a flexible linker, *e.g.*: GGGPGGG (SEQ ID NO:15); and

a Pvs28 sequence (with or without signal sequence or anchor):

AKVTAETQCKNGYVVQMSNHFECKCNDGFVMANENTCEEKRDCTNPQNVNKNCGDY
AVCANTRMNDEERALRCGCLGYTVMNEVCTPNKCNGVLCGKGKCILD PANVNSTM
SCNIGTTLDESKKCGKPGKTECTLKCKANEECKETQNYYKCVAKGSGGEGSGGEGSGG
EGSGGEGSGGEGSGGDTGAAYSLMN (SEQ ID NO:21).--

Please **delete** the following paragraph inserted in the amendment dated December 29, 2003 at page 38, line 23.

--In another embodiment, the Pvs25 or Pvs28 to be expressed can also be fused at the amino terminal end to the secretion signal sequence of the yeast mating pheromone alpha-factor (MF alpha 1S) and fused at the carboxy terminal end to the alcohol dehydrogenase II gene terminator (ADH2T), see van Rensburg (1997) J. Biotechnol. 55:43-53. The yeast alpha mating pheromone signal sequence allows for secretion of the expressed Pvs25 or Pvs28. In one embodiment, sequences are added after the KEX-2 cleavage site to enhance cleavage of the alpha factor leader; preferred embodiments include addition of the sequence EAEA (SEQ ID NO:22) and EAEAEAEAK (SEQ ID NO:23).--

Please **replace** the paragraph at **page 36, lines 14-21**, with the following:

--In another embodiment, the Pvs25 or Pvs28 to be expressed can also be fused at the amino terminal end to the secretion signal sequence of the yeast mating pheromone alpha-factor (MF alpha 1S) and fused at the carboxy terminal end to the alcohol dehydrogenase II gene terminator (ADH2T), see van Rensburg (1997) J. Biotechnol. 55:43-53. The yeast alpha mating

pheromone signal sequence allows for secretion of the expressed Pvs25 or Pvs28. In one embodiment, sequences are added after the KEX-2 cleavage site to enhance cleavage of the alpha factor leader; preferred embodiments include addition of the sequence EAEA (SEQ ID NO:22) and EAEAEAEAK (SEQ ID NO:23).--

Please **delete** the following paragraph inserted in the amendment dated December 29, 2003 at page 44, line 22.

--A highly conserved nucleotide sequence in the first EGF-like domain was identified. This sequence was used to synthesize a degenerate PCR oligonucleotide. To prevent the re-amplification of Pvs28 gene, nucleotides were chosen that were not identical to the Pvs28 sequence. A sense primer[[:]] (5'-GG(AT) TTT (CT)T(AG) (AG)(CT)T CA(AG) ATG AGT-3') (SEQ ID NO:6) was constructed. Using this primer with a vector-specific M13 universal primer (5'-GTA AAA CGA CGG CCA GT-3') (SEQ ID NO:7), nucleic acid sequences were amplified from a *P. vivax* genomic library (a *P. vivax* (Sal1) genomic library: Sau3AI partial digest cloned into pUC18 BamHI/BAP). The PCR reaction was: 94°C for 10 min, then 30 cycles of 94°C for 30 seconds, 44°C for 60 seconds and 72 °C for 2 min 30 seconds, and finally 72 °C for 8 min.--

Please **replace** the paragraph at **page 41, line 31 through page 42, line 6**, with the following:

--A highly conserved nucleotide sequence in the first EGF-like domain was identified. This sequence was used to synthesize a degenerate PCR oligonucleotide. To prevent the re-amplification of Pvs28 gene, nucleotides were chosen that were not identical to the Pvs28 sequence. A sense primer[[:]] (5'-GG(AT) TTT (CT)T(AG) (AG)(CT)T CA(AG) ATG AGT-3') (SEQ ID NO:6) was constructed. Using this primer with a vector-specific M13 universal primer (5'-GTA AAA CGA CGG CCA GT-3') (SEQ ID NO:7), nucleic acid sequences were amplified from a *P. vivax* genomic library (a *P. vivax* (Sal1) genomic library: Sau3AI partial digest cloned

into pUC18 BamHI/BAP). The PCR reaction was: 94°C for 10 min, then 30 cycles of 94°C for 30 seconds, 44°C for 60 seconds and 72 °C for 2 min 30 seconds, and finally 72 °C for 8 min.--

Please **delete** the following paragraph inserted in the amendment dated December 29, 2003 at page 45, line 31.

--After the purification of the individual amplified DNA fragments, each DNA fragment was cloned into pCR2.1 (Invitrogen) and by using plasmid-specific sequencing primers, eight individual recombinant plasmid clones were completely sequenced (ABI PRISM 310 Genetic Analyzer; PE Applied Biosystems). The full length open reading frame of Pvs25 gene sequence (SEQ ID NO:3) was obtained from this sequence these sequences, and the polypeptide sequence (SEQ ID NO:4) encoded therein deduced (Figures 1 and 2, SEQ ID NO:3 and SEQ ID NO:4, respectively) (Figures 3 and 4, respectively).--

Please **replace** the paragraph at **page 43, lines 5-10**, with the following:

--After the purification of the individual amplified DNA fragments, each DNA fragment was cloned into pCR2.1 (Invitrogen) and by using plasmid-specific sequencing primers, eight individual recombinant plasmid clones were completely sequenced (ABI PRISM 310 Genetic Analyzer; PE Applied Biosystems). The full length open reading frame of Pvs25 gene sequence (SEQ ID NO:3) was obtained from this sequence these sequences, and the polypeptide sequence (SEQ ID NO:4) encoded therein deduced (Figures 1 and 2, SEQ ID NO:3 and SEQ ID NO:4, respectively) (Figures 3 and 4, respectively).--

Please **delete** the following paragraph inserted in the amendment dated December 29, 2003 at page 46, line 28.

--The resultant nucleic acid construct encoding a Pvs25 fusion protein (SEQ ID NO:16) was ligated into the NheI and ApaI restriction sites of the yeast shuttle vector, YepRPEU-3, as schematically represented in Figure 6. Recombinant clones were electroporated into the host *S. cerevisiae* strain, VK1, and clones harboring the recombinant plasmid were screened for their ability to secrete a His6 (SEQ ID NO:24) tagged protein. A single high-producing colony was amplified in selective growth media and was used to establish a cell bank for yeast expressed Pvs25.--

Please **replace** the paragraph at page 43, line 32 through page 44, line 4, with the following:

--The resultant nucleic acid construct encoding a Pvs25 fusion protein (SEQ ID NO:16) was ligated into the NheI and ApaI restriction sites of the yeast shuttle vector, YepRPEU-3, as schematically represented in Figure 6. Recombinant clones were electroporated into the host *S. cerevisiae* strain, VK1, and clones harboring the recombinant plasmid were screened for their ability to secrete a His6 (SEQ ID NO:24) tagged protein. A single high-producing colony was amplified in selective growth media and was used to establish a cell bank for yeast expressed Pvs25.--

Please **delete** the following paragraph inserted in the amendment dated December 29, 2003 at page 48, line 5.

--The resultant nucleic acid construct encoding a Pvs28 fusion protein (SEQ ID NO:17) was ligated into the NheI and ApaI restriction sites of the yeast shuttle vector, YepRPEU-3 (as schematically represented in Figure 6). Recombinant clones were

electroporated into the host *S. cerevisiae* strain, VK1, and clones harboring the recombinant plasmid were screened for their ability to secrete a His6 (SEQ ID NO:24) tagged protein. High-producing colonies were amplified in selective growth media and used to establish cell banks for yeast expressed recombinant Pvs28.--

Please **replace** the paragraph at **page 45, lines 5-11**, with the following:

--The resultant nucleic acid construct encoding a Pvs28 fusion protein (SEQ ID NO:17) was ligated into the NheI and ApaI restriction sites of the yeast shuttle vector, YepRPEU-3 (as schematically represented in Figure 6). Recombinant clones were electroporated into the host *S. cerevisiae* strain, VK1, and clones harboring the recombinant plasmid were screened for their ability to secrete a His6 (SEQ ID NO:24) tagged protein. High-producing colonies were amplified in selective growth media and used to establish cell banks for yeast expressed recombinant Pvs28.--

Please **delete** the following paragraph inserted in the amendment dated December 29, 2003 at page 48, line 14.

--For expression in yeast, a Pvs28 DNA fragment was generated, as described above. The nucleic acid was modified to encode a glutamine, rather than an asparagine, at amino acid residue number 130 (see Figure 7 Figure 6, "Pvs28Q130"). A polyhistidine tag sequence was spliced into the polypeptide coding sequence.--

Please **replace** the paragraph at **page 45, lines 14-17**, with the following:

--For expression in yeast, a Pvs28 DNA fragment was generated, as described above. The nucleic acid was modified to encode a glutamine, rather than an asparagine, at amino acid residue number 130 (see Figure 7 Figure 6, "Pvs28Q130"). A polyhistidine tag sequence was spliced into the polypeptide coding sequence.--

Please **delete** the following paragraph inserted in the amendment dated December 29, 2003 at page 48, line 18.

--The resultant nucleic acid construct encoding this modified (partially deglycosylated) Pvs28 fusion protein (SEQ ID NO:17 "Pvs28Q130"; SEQ ID NO:18) was ligated into the NheI and ApaI restriction sites of the yeast shuttle vector, YepRPEU-3 (as schematically represented in Figure 6). Recombinant clones were electroporated into the host *S. cerevisiae* strain, VK1, and clones harboring the recombinant plasmid were screened for their ability to secrete a His6 (SEQ ID NO:15) tagged protein. High-producing colonies were amplified in selective growth media and used to establish cell banks for yeast-expressed recombinant Pvs28.--

Please **replace** the paragraph at **page 45, lines 18-24**, with the following:

--The resultant nucleic acid construct encoding this modified (partially deglycosylated) Pvs28 fusion protein (SEQ ID NO:17 "Pvs28Q130"; SEQ ID NO:18) was ligated into the NheI and ApaI restriction sites of the yeast shuttle vector, YepRPEU-3 (as schematically represented in Figure 6). Recombinant clones were electroporated into the host *S. cerevisiae* strain, VK1, and clones harboring the recombinant plasmid were screened for their ability to secrete a His6 (SEQ ID NO:15) tagged protein. High-producing colonies were amplified in selective growth media and used to establish cell banks for yeast-expressed recombinant Pvs28.--